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Nanoscale imaging and characterization of *Caenorhabditis elegans* epicuticle using atomic force microscopy

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Abstract

Here we introduce PeakForce Tapping non-resonance atomic force microscopy for imaging and nanomechanical mapping of *Caenorhabditis elegans* nematodes. The animals were imaged both in air and water at nanoscale resolution. Layer-by-layer glass surface modification was employed to secure the worms for imaging in water. Microtopography of head region, annuli, furrows, lateral alae and tail region was visualized. Analysis of nanoscale surface features obtained during AFM imaging of three larval and adult hermaphrodite nematodes in natural environment allowed for numerical evaluation of annuli periodicity, furrows depth and annuli roughness. Nanomechanical mapping of surface deformation, Young modulus and adhesion confirms that the mechanical properties of the nematode cuticle are non-uniform. Overall, PeakForce Tapping AFM is a robust and simple approach applicable for nanoscale three-dimensional imaging and characterization of *C. elegans* nematodes.

Key words: Atomic force microscopy; AFM; *Caenorhabditis elegans*; PeakForce Tapping

Nematodes have attracted enormous research efforts due to their remarkable biology and the role they play in human life. Nematodes, both free-living and parasitic, are known to populate most known habitats on Earth, including high temperature 3-kilometer-deep subsurface.[1] Numerous nematode species pose a serious threat to human health as parasites[2,3] or affecting severely agriculture as animal parasites and ubiquitous plant pests.[4] In addition, nematodes have been intensively used in general biomedical research as very convenient model organisms. For instance, the free-living soil nematode *Caenorhabditis elegans* has become one of the most recognized and praised experimental model animals in biomedical research.[5] This tiny worm has been successfully employed to elucidate the molecular mechanisms of RNA interference,[6] aging research,[7–9] neuromuscular disorders investigations,[10] behavioral assays[11] and microbiology.[12] In addition, drug discovery,[13] toxicology[14] and nanomaterials toxicity[15–17] studies have been successfully performed using *C. elegans* model.

One of the reasons for the tremendous success of the *C. elegans* experimental model was the fact that this transparent animal can be easily imaged with routine optical microscope,[18] making it very easy to visualize its internal organs, tissues, single cells and even subcellular events. Consequently, numerous papers exist demonstrating applications of microscopy methods to investigate the processes inside the nematodes. In addition, the microscopic investigation of the cuticle of *C. elegans* microworms offers a powerful tool for diagnosis of nematode well-being. Normally, the conventional methodology of *C. elegans* specimen preparation for transmission (TEM) and scanning (SEM) electron microscopies relies on embedding the worms into solidified resins and coating the samples with a conductive thin layer, respectively. Fixation and contrast staining may damage or alter the cuticle structure. More sophisticated methods, such as freeze-fracture replica preparation[19] are time and labor-consuming. Although electron microscopy allows for

Abbreviations: AFM, atomic force microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy.

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